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For Research Use Only.

This mouse prolactin antigen assay is intended for the quantitative determination of prolactin antigen in mouse plasma.

Mouse prolactin (PRL) is a 197 aa, 23kD peptide hormone [1] that is secreted primarily by the pituitary gland in both males and females, though its major roles are in pregnancy and lactation [2,3]. Prolactin may have a role in breast cancer development, with higher prolactin levels correlating with postmenopausal breast cancer risk [4].

Mouse prolactin will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, biotinlyated anti-mouse prolactin primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody, which is proportional to the total prolactin present in the samples, is reacted with peroxidase-conjugated streptavidin. Following an additional washing step, TMB substrate is used for color development which is measured at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of mouse prolactin. Color development is proportional to the concentration of prolactin in the samples.

- **96-well microtiter strip plate**
  8X12 well removable strips containing affinity purified anti-mouse prolactin antibody on the surface. Strips are blocked and dried.
- **10X Wash buffer**
  1 bottle of 50ml; bring to 1X using DI water
- **Mouse prolactin standard**
  1 vial of lyophilized standard
- **Anti-mouse prolactin primary antibody**
  1 vial of lyophilized biotinylated antibody
- **Peroxidase conjugated streptavidin**
  1 vial of concentrated streptavidin-HRP
- **TMB substrate solution**
  1 bottle of 10ml solution

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -70°C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

- 1-channel pipettes covering 0-10µl and 200-1000µl
- 12-channel pipette covering 30-300µl
- Paper towels or kimwipes
- 50ml tubes, 1.5ml centrifuge tubes
- 1N H_2SO_4
- DI water
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

Warning – Avoid skin and eye contact when using TMB substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

- **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- **DO NOT** pipette reagents by mouth.
- Always pour TMB substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the TMB substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

- **TBS buffer:** 0.1M Tris 0.15M NaCl pH 7.4
- **Blocking buffer (BB):** 3% BSA in TBS
- **Wash buffer concentrate:** The wash buffer is supplied in a 10X concentrate and must be diluted 1:10 with deionized water for use with the kit.

The assay measures total mouse prolactin in the 0.1-100ng/ml range. Samples with prolactin levels above 100ng/ml should be diluted in blocking buffer before use. Use of straight or a 1:2 dilution for normal plasma is suggested to ensure that resulting values fall within the linear range of the assay. Optimal dilutions should be experimentally determined by the researcher.

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard:
Reconstitute standard as directed on the vial to give a 500ng/ml solution.

Dilution table for preparation of mouse prolactin standard:

Prolactin concentration (ng/ml)	Dilutions
100	800µl (BB) + 200µl (500ng/ml)
50	500µl (BB) + 500µl (100ng/ml)
25	500µl (BB) + 500µl (50ng/ml)
10	600µl (BB) + 400µl (25ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2.5	500µl (BB) + 500µl (5ng/ml)
1	600µl (BB) + 400µl (2.5ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0.25	500µl (BB) + 500µl (0.5ng/ml)
0.1	600µl (BB) + 400µl (0.25ng/ml)
0	500µl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition:
Remove microtiter plate from bag. Add 100µl standards in duplicate and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Primary Antibody Addition:
Add 10ml of blocking buffer directly to the primary antibody vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

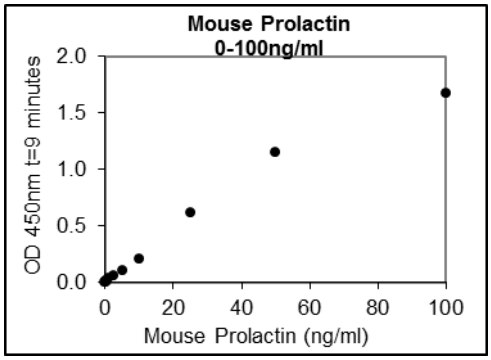
Streptavidin-HRP Addition:
Dilute 1µl into 10ml of blocking buffer and mix well. Add 2.5ml of the diluted secondary into 7.5ml of blocking buffer (for a final dilution of 1:40,000) and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

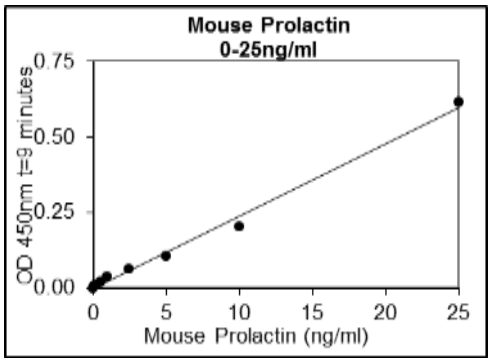
Substrate Incubation:
Add 100µl TMB substrate to all wells and shake plate for 6-12 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly and read final absorbance values at 450nm. For best results read plate immediately

Measurement:
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Assay Calibration:
Plot A_{450} against the amount of mouse prolactin in the standards. Fit a straight line through the points using a linear fit procedure. The amount of total mouse prolactin in the unknowns can be determined from this curve.

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





The concentration of prolactin in pooled normal mouse plasma determined by in-house testing was 3-5ng/ml. Prolactin levels are elevated in pregnant mice and peak at day 8 of pregnancy [5].

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling or from contact with the above product.

1. Kohmoto K *et al*. Complete amino acid sequence of mouse prolactin. Eur J Biochem. 1984; 138:227-237.
2. Goffin V *et al.* Prolactin: the new biology of an old hormone. Annu Rev Physiol. 2002; 64:47-67.
3. Shiu RP and Friesen HG. Mechanism of action of prolactin in the control of mammary gland function. Annu Rev Physiol. 1980; 42:83-96.
4. Hankinson SE *et al*. Plasma prolactin levels and subsequent risk of breast cancer in postmenopausal women. J Natl Cancer Inst. 1999; 91:629-634.
5. Murr S *et al*. Plasma luteinizing hormone, follicle-stimulating hormone and prolactin during pregnancy in the mouse. Endocrinology 1974; 94:112-116.

Example of ELISA Kit Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.1ng/ml	0.25ng/ml	0.5ng/ml	1ng/ml	2.5ng/ml	5ng/ml	10ng/ml	25ng/ml	50ng/ml	100ng/ml	
B	0	0.1ng/ml	0.25ng/ml	0.5ng/ml	1ng/ml	2.5ng/ml	5ng/ml	10ng/ml	25ng/ml	50ng/ml	100ng/ml	
C												
D												
E												
F												
G												
H												

96 Well Plate
Standards: 22 Wells
Samples: 74 Wells